REMARKS

Claims 14 and 17-42 remain in this application. New claims 43 and 44 are added. Claims 14, 17, 18, and 35 have been amended. The amendments to the claims have been made to further prosecution. Support for the amendment to claim 14 can be found in Figures 6A, 6B, and 6C and paragraphs 0025, 0026, 0027, and 0075. Support for new claims 43 and 44 can be found in the application as filed, at least at Figures 6A, 6B, and 6C and paragraphs 0102, 0103 and 0111. No new matter has been added.

Paragraph 0102 of the specification has been amended to correct an obvious error, the omission of the volume of the transfection mixture. This correction of the error would be clear to one of ordinary skill in the art by the reference to the "same conditions for Lipofectamine™ transfection" and the description in paragraph 0111. A copy of the manufacturer's instructions for the use of Lipofectamine™ for the transfection of siRNA, specifying a volume of 1 ml for a 60 mm culture dish is provided with a Supplementary Information Disclosure Statement submitted herewith. See also Declaration of Tariq M. Rana Under 37 C.F.R.§1.132, submitted herewith.

Objections to the Claims

Claims 35-37 were objected to under 37 CFR §1.75(c) as being in improper form because a multiple dependent claim should refer to other claims in the alternative only and/or cannot depend from any other multiple dependent claim. Claim 35 has been amended to be dependent on claims 24-32, and is thus no longer improperly dependent on a multiple dependent claim. Claims 36 and 37 are singly dependent on claim 35, which is now in proper form. Applicant respectfully requests that the objection to claims 35-37 be reconsidered in view of the amendment of claim 35, and treated on the merits.

Claims 17 and 18 were objected to under 37 CFR §1.75(c) as being in improper dependent form for failing to further limit the subject matter of a previous claim. Claims 17 and 18 have been amended to place the claims in proper dependent form.

Claim Rejections

Claim Rejection Under 35 U.S.C. § 102(e)

Claims 14, 19-20, 23-24, 28-29 and 31-34 stand rejected under 35 U.S.C. § 102(e) as being anticipated by Woolf (US2006/0009409). Applicant submits that Woolf neither teaches nor suggests the present claimed invention.

Dendrimers are generally characterized as having three component parts: a central core, an interior dendritic structure, and an exterior surface. An exemplary dendrimer, poly(amidoamine) (PAMAM), is synthesized by a two step strategy, with each iteration of the two step sequence termed a "generation," where the core is regarded as generation 0, and the product of the first two-step cycle is generation 1. See Stevens, M.P., *Polymer Chemistry. An Introduction, 3rd Ed.,* pp. 301-303, Oxford University Press, New York 1999. Typically, cores of PAMAMs include ammonia, which yields three starting arms, cystamine or ethylenediamine, each of which yields four starting arms. See Tomalia, D.A., Birth of a New Macromolecular Architecture: Dendrimers as Quantized Building Blocks for Nanoscale Synthetic Organic Chemistry, Aldrichimica Acta, 37(2): 39-57, 2004.

Table 1
Physical Characteristics of PAMAM Dendrimers (EDA Core)
From D'Emanuele, A., et al., "Dendrimers"pp.1-21 in Encyclopedia of Pharmaceutical Technology, Taylor & Francis 2003

Generation	Molecular Wt.	Measured Diameter (nm)	Number of Surface Groups
0	517	1.5	4
1	1,430	2.2	8
2	3,256	2.9	16
3	6,909	3.6	32
4	14,215	4.5	64
5	28,826	5.4	128
6	58,048	6.7	256
7	116,493	8.1	512
8	233,383	9.7	1024
9	467,162	11.4	2048
10	934,720	13.5	4096

Table 1, above, illustrates the increase in molecular weight, diameter and number of surface groups with increasing generations of PAMAM dendrimers based on a ethylenediamine core. Comparing the generation 10 to the generation 2 dendrimers, there is a 287 fold difference in molecular weight, 4.7 fold difference in diameter and a 256 fold difference in the number of surface groups.

There are crucial differences in three-dimensional structure that accompany these substantial differences in molecular weight and size that are encompassed in the broad term "PAMAM dendrimers." Figure 4 of Svenson & Tomalia (2005), below, shows PAMAM dendrimers of generation 1-3 as flexible open dendritic structures, PAMAM dendrimers of generation 4-6 as spherical dendritic structures having container properties with an accessible interior, and flexible open dendritic structures, and PAMAM dendrimers of generation 7-10 as spherical dendritic structures having a rigid surface structure and an inaccessible interior. Direct transmission electron microscopy of generation 5-10 PAMAM dendrimers (Figure 9 of Tomalia, D.A., Birth of a New Macromolecular Architecture: Dendrimers as Quantized Building Blocks for Nanoscale Synthetic Organic Chemistry, Aldrichimica Acta, 37(2):39-57, 2004, below) shows them to be particles, with sizes consistent with those predicted in Table 1 and a nearly monodisperse distribution of diameters. The particulate nature of siRNA/PAMAM dendrimers has recently been confirmed by direct observation (See below, Figure 1C of Zhou, J., et al., PAMAM dendrimers for efficient siRNA delivery and potent gene silencing, Chem Commun (Camb). 2006 Jun 14;(22):2362-4)

The pending claims are directed to a delivery mixture comprising a generation 2 to 5 dendrimer and a nucleic acid capable of mediating RNA interference. The Woolf reference neither teaches nor suggests a delivery mixture comprising a generation 2 to 5 dendrimer and a nucleic acid capable of mediating RNA interference.

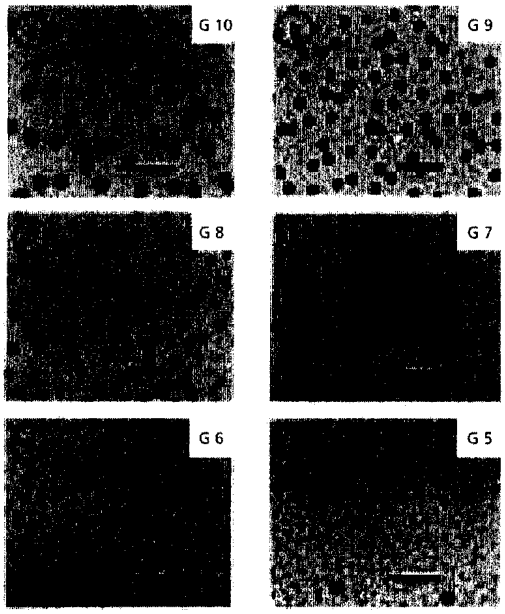

Figure 9. Transmission Electron Micrographs (TEMs) of Gen 5–10 PAMAM Dendrimers. Sample ⓕ Contains Three Molecules of Gen 10 Dendrimer for Comparison. Bar Length = 50 nm. (Reproduced from Reference 80 with Permission from ACS.)

Figure 9 of Tomalia, D.A., Birth of a New Macromolecular Architecture: Dendrimers as Quantized Building Blocks for Nanoscale Synthetic Organic Chemistry, Aldrichimica Acta, 37(2):39-57, 2004

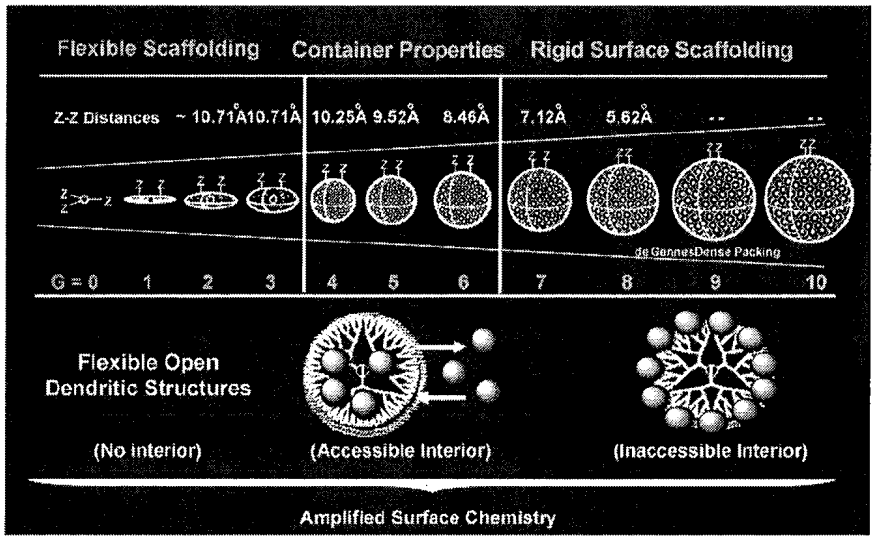

Figure 4 of Svenson, S., & Tomalia, D.A., Dendrimers in biomedical applications--reflections on the field, Adv Drug Deliv Rev. 2005 Dec 14;57(15):2106-2129, showing the periodic properties of PAMAM dendrimers generations G = 0-10 depicting the distances between surface charges (Z-Z), including the 'de Gennes dense packing', the generation-dependent size and shape if dendrimers, and their 'nanoscale-container' and 'nanoscaffolding' properties.

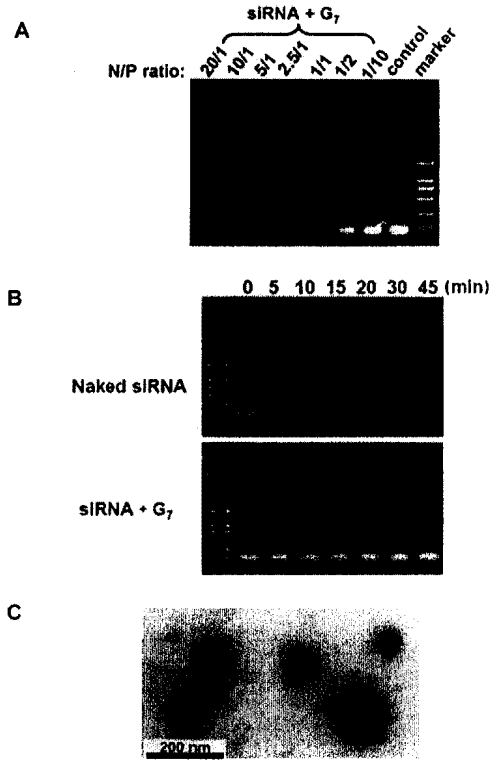

Fig. 1 (A) Agarose gel analysis of the siRNA–dendrimer complexes. Migration of the siRNA/G_7 complexes at charge ratios N/P varying from 1/10 to 20/1 in Tris–HCl buffer solution (pH 7.6). (B) Dendrimer G_7 is able to prevent siRNA from RNase A degradation. Naked siRNA and siRNA/G_7 complex (N/P 2.5/1) were incubated in the presence of 0.01 μg/μL RNase A for 0, 5, 10, 15, 20, 30 and 45 min at 37 °C and then agarose gel electrophoresis was performed. (C) TEM imaging of the siRNA/G_7 complex prepared in Tris–HCl buffer, pH 7.6.

Zhou, J., et al., PAMAM dendrimers for efficient siRNA delivery and potent gene silencing, Chem Commun (Camb). 2006 Jun 14;(22):2362-4. Epub 2006 May 10

The Woolf reference does not teach or suggest which of the different PAMAM dendrimers would be suitable for use in a delivery mixture comprising a dendrimer and a nucleic acid capable of mediating RNA interference (RNAi). The sole mention of PAMAM dendrimers in Woolf is once in paragraph 203, where it is one of 14 listed cationic lipids. There is no teaching to choose one or more classes or generations of PAMAM dendrimers. This single mention is not an enabling disclosure to one of ordinary skill in the art, but is, at best, an invitation to experiment. The rejection of claims 14, 19-20, 23-24, 28-29 and 31-34 under 35 U.S.C. § 102(e) as being anticipated by Woolf (US2006/0009409) is unwarranted, and should be withdrawn.

Claim Rejections Under 35 U.S.C. § 103(a)

Claims 14, 19-20, and 23-34 stand rejected under 35 USC § 103(a) as being unpatentable over Woolf (US2006/0009409) in view of Olejnik et al. (Nucleic Acids Research, 1996) in view of Grigoriev et al. (PNAS 1993). As noted above, the Woolf reference does not teach or suggest which of the PAMAM dendrimers would be suitable for use in a delivery mixture comprising a dendrimer and a nucleic acid capable of mediating RNA interference (RNAi).

The Olejnik reference discloses the design, synthesis and evaluation of a non-nucleosidic photocleavable biotin phosphoramidite (PCB-phosphoramidite) that provides a simple method for purification and phosphorylation of oligonucleotides (abstract). The Olejnik reference neither teaches nor suggests a delivery mixture comprising a generation 2 to 5 dendrimer and a nucleic acid capable of mediating RNA interference.

The Grigoriev reference does not teach or suggest a delivery mixture comprising a generation 2 to 5 dendrimer and a nucleic acid capable of mediating RNA interference. Rather, the Grigoriev reference discloses the use of psoralen-oligonucleotide conjugates that are transfected into cells by electroporation, and which, upon binding to double-stranded DNA sequences via triple helix formation, may be cross-linked *in vitro* to both strands of the DNA following UV irradiation (abstract, page 3502, left column, last full paragraph).

The addition of the Olejnik reference and the Grigoriev reference does not cure the lack of teaching regarding PAMAM dendrimers suffered by the Woolf reference. Thus the

combination of Woolf with Olejnik and Grigoriev does not teach or suggest the present claimed invention. The rejection of claims 14, 19-20, and 23-34 under 35 USC § 103(a) as being unpatentable over Woolf (US2006/0009409) in view of Olejnik et al. (Nucleic Acids Research, 1996) in view of Grigoriev et al. (PNAS 1993) is unwarranted, and should be withdrawn.

Claims 14, 17-24, 32-34, and 38-42 stand rejected under 35 USC § 103(a) as being unpatentable over Yoo et al. in view of Hammond et al. (Nature 2001, 2:110-119), Tuschl et al. (WO 02/44321) and McManus et al. (Nature Review: Genetics 2002).

The Office Action characterizes Yoo et al. as teaching a delivery mixture comprising a PAMAM dendrimer and an antisense nucleic acid capable of inhibiting gene expression. Yoo et al. state that they used generation 4, 5, and 7 PAMAM dendrimers with a core of ethylenediamine. However, Yoo et al. take the position that the complexes of antisense RNA and dendrimers are not particles, but are soluble macromolecules, since the supernatants after ultracentrifugation were capable of inducing protein expression in their assay. Yoo et al. speculate with no direct evidence that their results could be due to complex formation between serum proteins, dendrimers and antisense oligonucleotides. However, their results show that the activation of their reporter in the absence of serum is *statistically the same as* the activation in the presence of 10% serum, the standard culture conditions (page 1800, left column, Figure 2B).

The interpretation by Yoo et al. that the complexes of antisense RNA and dendrimers are not particles is a direct contradiction of clear evidence in the art that such complexes formed with PANAM dendrimers are particles. See Figure 9 of Tomalia, D.A., Birth of a New Macromolecular Architecture: Dendrimers as Quantized Building Blocks for Nanoscale Synthetic Organic Chemistry, Aldrichimica Acta, 37(2):39-57, 2004, above for a direct demonstration of the particulate character. The particulate nature of siRNA/PAMAM dendrimer complexes has recently been confirmed by direct observation (See above, Figure 1C of Zhou, J., et al., PAMAM dendrimers for efficient siRNA delivery and potent gene silencing, Chem Commun (Camb). 2006 Jun 14;(22):2362-4; and Lee, J.H., et al., Quaternized Polyamidoamine Dendrimers as Novel Gene Delivery System: Relationship between Degree of Quaternization and their influences, Bulletin of the Korean Chemical Society, 2003, 24(11) 1637-1640). The fact that Yoo et al. take the position that their delivery mixture is not particulate, contrary to the

weight of evidence in the art, is a strong factor against combining the Yoo et al. reference with the other cited references.

Failure of others is one of the secondary considerations, or indicia of nonobviousness. Graham v. John Deere Co., 383 U.S. 1, 17-18, 148 USPQ 459, 467 (1966). Here, Yoo et al. state that a "generation 4 dendrimer was relatively ineffective under all conditions." In contrast, the present claimed invention is quite effective with a generation 4 dendrimer, in fact, comparable to Lipofectamine™ in the concentration range of 20-40 μg/ml of dendrimer. See paragraphs 0102 and 0103 and Figs. 1 and 2 of the present application as well as the Declaration of Tariq M. Rana Under 37 C.F.R.§1.132. The failure of Yoo et al. to transfect cells with a generation 4 dendrimer indicates that the present claimed invention should not be rejected as obvious over Yoo et al. in view of Hammond et al. (Nature 2001, 2:110-119), Tuschl et al. (WO 02/44321) and McManus et al.(Nature Review: Genetics 2002). The Hammond, Tuschl and McManus references all are used to support the argument that one of ordinary skill would substitute dsRNA, siRNA or miRNA for the antisense oligonucleotides of Yoo et al. The combination of the Hammond, Tuschl and McManus references with the Yoo et al. reference would not teach or suggest delivery mixture comprising a generation 2 to 5 dendrimer and a nucleic acid capable of mediating RNA interference.

With regards to the substitution of siRNA for the antisense oligonucleotides of Yoo et al., at best, the Office Action is advancing an "obvious to try" argument. "An 'obvious-to-try' situation exists when a general disclosure may pique the scientist's curiosity, such that further investigation might be done as a result of the disclosure, but the disclosure itself does not contain a sufficient teaching of how to obtain the desired result, or that the claimed result would be obtained if certain directions were pursued." In re Eli Lilly & Co., 902 F.2d, 943, 945 (Fed Cir. 1990). However, "obvious to try is not the standard," what is required is a "reasonable expectation of success". In re O'Farrell, 853 F.2d 894, 904 (Fed. Cir.1988).

Those who have tried the substitution of siRNA for the antisense oligonucleotides of Yoo et al., as suggested in the Office Action, have *not* demonstrated that success is likely. Kang, H. et al. have reported that dendrimer-oligonucleotide complexes were moderately effective for delivery of antisense and only poorly effective for the delivery of siRNA (cited below). When

compared to transfection using Lipofectamine 2000 as a positive control, dendrimers were about as effective in producing reduction of gene expression using antisense, but much less effective in producing reduction of gene expression with siRNA. Kang, H. et al., Tat-conjugated PAMAM dendrimers as delivery agents for antisense and siRNA oligonucleotides, Pharm Res. 2005 Dec; 22(12):2099-106. In contrast, as noted above, the present claimed invention is comparable to Lipofectamine™ in the concentration range of 20-40 μg/ml of dendrimer.

The present invention also provides unexpected results, both quantitatively and qualitatively, that are not taught or suggested by the combination of the Hammond, Tuschl and McManus references with the Yoo et al. reference. The present invention discloses that dendrimer concentrations above 40 μg/ml are less effective in producing cell uptake and RNAi (Figs. 1 and 2) and that the localization of the siRNA at higher concentration of dendrimer is different than produced by either Lipofectamine™ or concentrations of 20-40 μg/ml. In contrast, the Yoo et al. reference alone or in combination with Hammond, Tuschl and McManus references indicates an increase in effect with increasing concentrations of dendrimer (Fig. 2a).

For the reasons stated above, the Applicant submits that the rejection of claims 14, 17-24, 32-34, and 38-42 under 35 USC § 103(a) as being unpatentable over Yoo et al. in view of Hammond et al., Tuschl et al., and McManus et al. is unwarranted, and withdrawal of the rejection is respectfully requested.

CONCLUSION

In light of the amendments and arguments presented herein, the Applicant respectfully submits that all pending claims are in condition for allowance and requests a timely Notice of Allowance to follow in this case. The Applicant requests that the Examiner telephone the undersigned at (508) 929-1658 in the event a telephone discussion would be helpful in advancing the prosecution of the present case.

Respectfully submitted,

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